Discovery and Nonclinical Characterization of a Novel CRFR2 Selective and Biased UCN2 Analog, HM17321, for High Quality Weight Management

Hanmi

Poster-504

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ABSTRACT

Background: Despite efficient weight loss, incretin therapy results in significant lean mass loss and impaired weight loss quality (WLQ). So, exploration of novel therapeutic targets for WLQ improvement is of great interest. Urocortin-2 (UCN2) has shown to induce lipolysis and muscle hypertrophy, suggesting its potential for WLQ improvement. However, fine-tuned receptor signaling is a critical step for desired outcomes. To aim this, structure-based approach was applied to rationally design a novel UCN2 analog. Here, we provide the nonclinical evidences of HM17321 for high quality weight management.

Method: CRFR-mediated cAMP accumulation and β-arrestin recruitment assay were conducted. In DIO mice, HM17321 was administered for 4 weeks, followed by body composition, muscle tissue and blood analysis. Semaglutide (Sema) was included as comparative control.

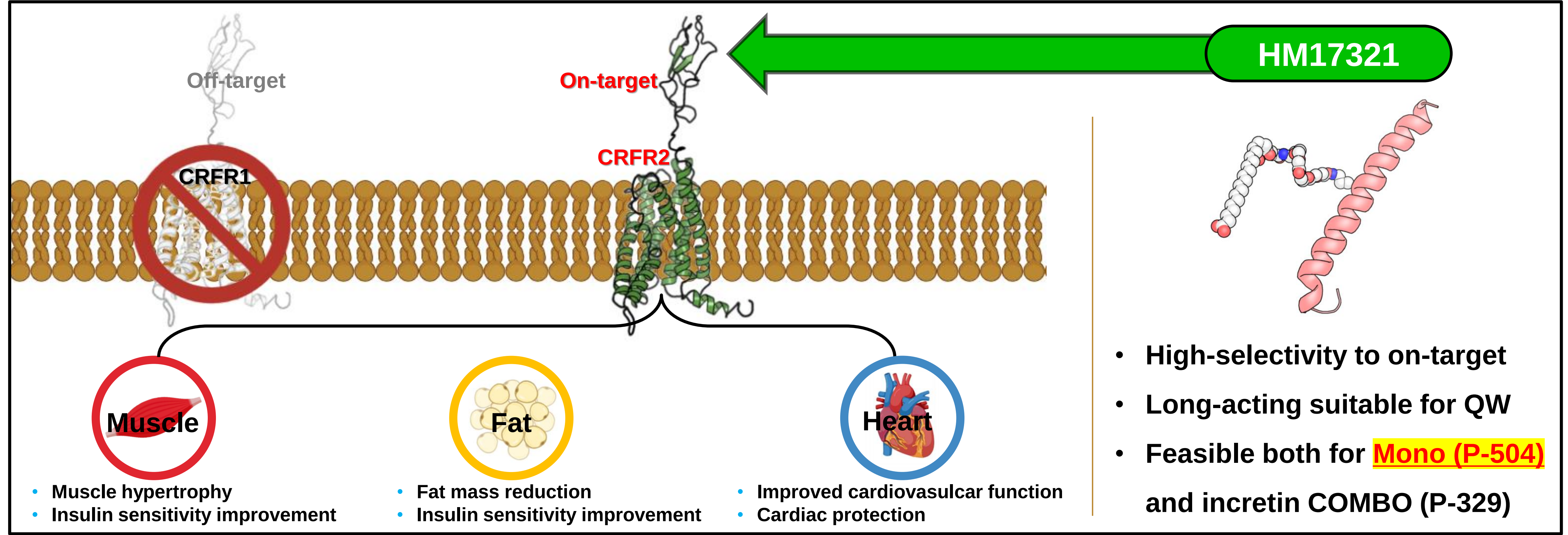
Results: Complex structure of UCN2:CRFR2 (corticotropin-releasing factor receptor 2) was derived by AlphaFold 2 for *in silico* analog design. Then, the interface was systematically analyzed by graph neural network to fine-tune the interaction between UCN2 analogs and CRFRs. According to comprehensive in vitro characterization, HM17321 showed unique properties such as CRFR2 selectivity and biased agonism. In DIO mice, HM17321 treatment led to fat mass loss driven weight loss (-17.3% *vs.* D0) comparable to Sema. Notably, unlike Sema, HM17321 not only increased lean mass, but also decreased lipid contents in skeletal muscle tissue, indicating quantitative and qualitative improvement effect of HM17321 on muscle tissue. Improvement effect on metabolic parameters was also confirmed.

Conclusion: HM17321, a novel long-acting CRFR2-selective and biased UCN2 analog, is capable of reducing fat mass and increasing lean mass in DIO mice. These data suggest that HM17321 deserves to be developed as stand-alone therapy as well as incretin COMBO for high quality weight management. Human relevance of these findings warrants further research.

BACKGROUND

The next generation obesity drugs : Is it possible to burn fat while gaining muscle?

HM17321, a novel long-acting CRFR2 selective and biased UCN2 analog, is optimally designed to selectively reduce fat while simultaneously increasing muscle mass during weight loss, maximizing WLQ and metabolic improvement.



- Muscle hypertrophy
- Insulin sensitivity improvement

- Fat mass reduction
- Insulin sensitivity improvement

- Improved cardiovasulcar function
- Cardiac protection

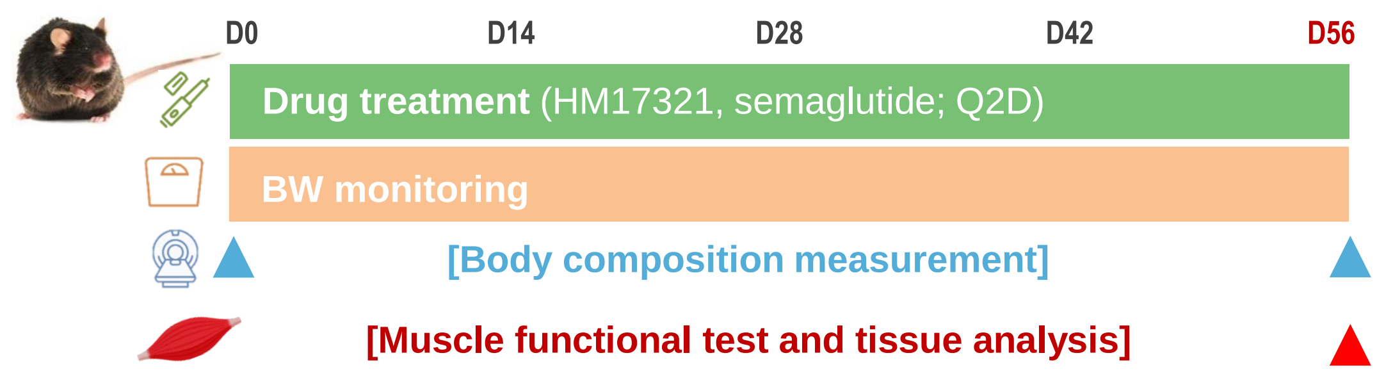
- **High-selectivity to on-target**
- **Long-acting suitable for QW**
- **Feasible both for Mono (P-504) and incretin COMBO (P-329)**

METHODS

Experimental scheme

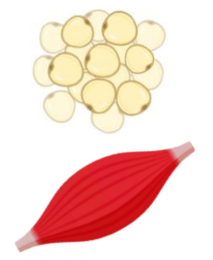
Study 1. *In vivo* study

DIO* mice, ♂ 20W 6-week-old, HFD for 14 weeks
(C57BL/6, n=6/group)



Note.
*DIO: (High-fat) Diet-Induced Obese

Study 2. *In vitro* study



1) 3T3-L1 murine preadipocyte cell line,
isolated from the embryo of a mouse

2) Primary Human Skeletal Muscle Cells (HSkMC),
isolated from normal, human skeletal muscle

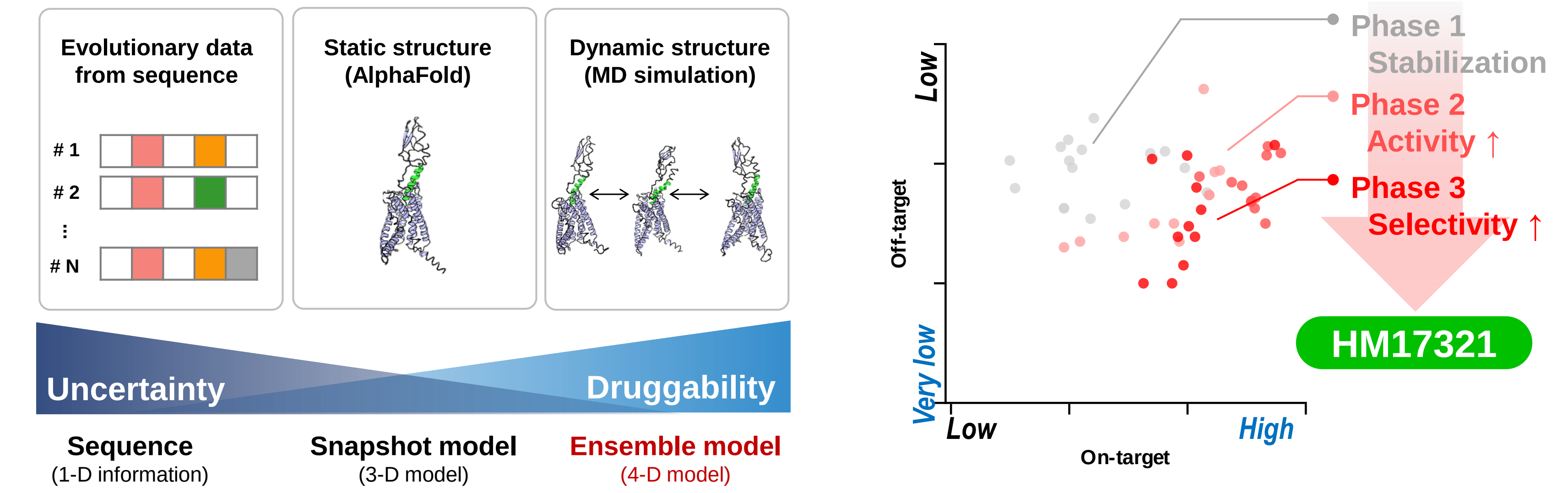
- To evaluate the effect of HM17321 (*vs.* Sema) on high quality weight loss (derived exclusively from fat mass), HM17321 (or Sema) was administered for 8 weeks (D56), body composition (minispec LF90II, Bruker) and wire hang test were assessed at D56, then followed by skeletal muscle tissue analysis [Study 1].
- To elucidate the direct MoAs of HM17321 for potent fat mass reduction and muscle hypertrophic effects observed *in vivo*, both 3T3-L1 preadipocytes and primary human skeletal muscle cells were utilized [Study 2].

RESULTS

Drug moiety design driven by *in silico* modeling

Figure 1. Design of Hanmi's novel drug moiety by *in silico* approach

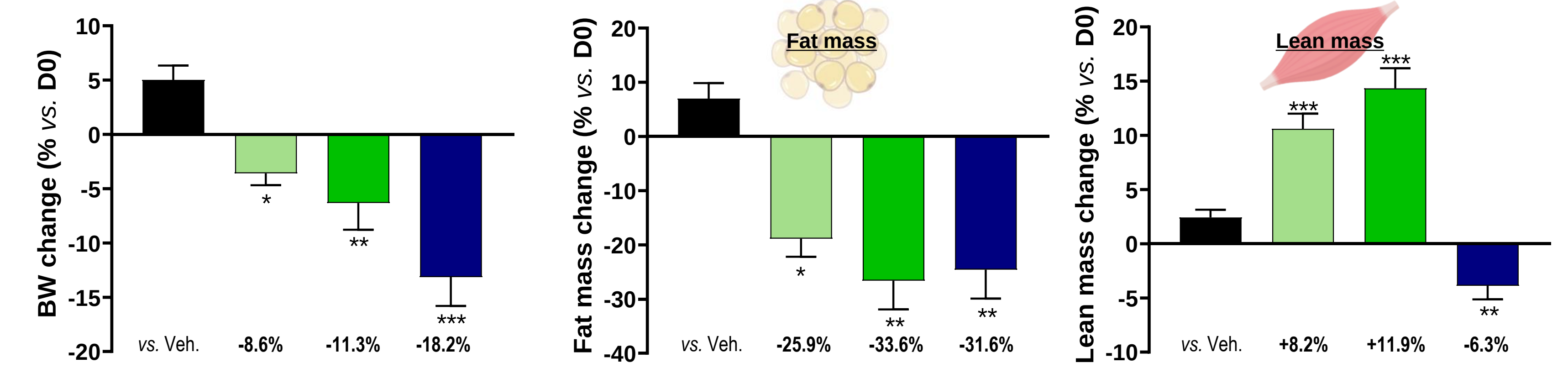
(a) Advancing with ensemble structural models (b) Design landscape



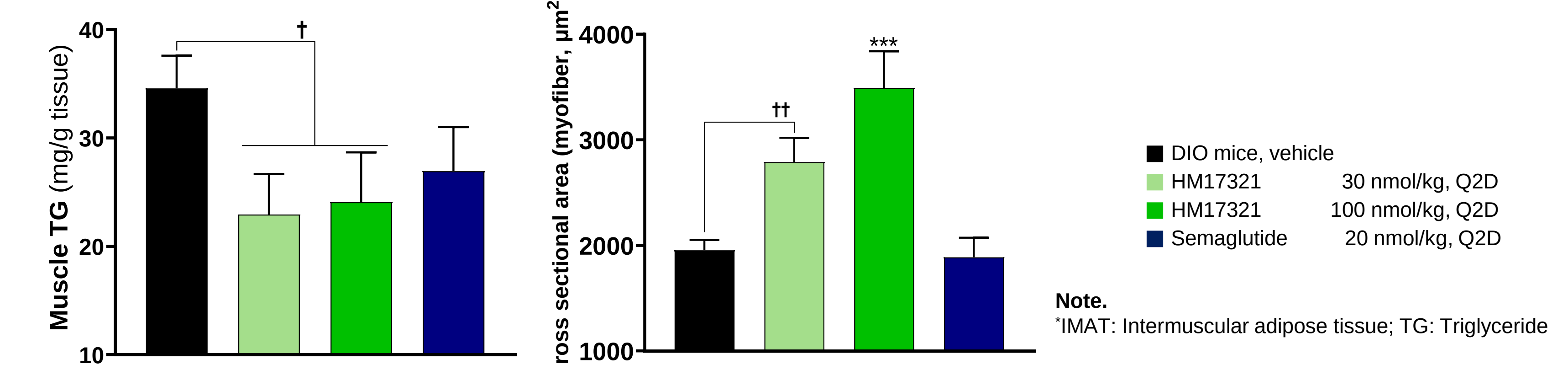
Body re-composition effect of HM17321 at D56 in DIO mice

Figure 2. Effect of HM17321 on BW, fat, lean mass, IMAT* levels, and myofiber

(a) Change in BW from BL (b) Change in body composition from BL



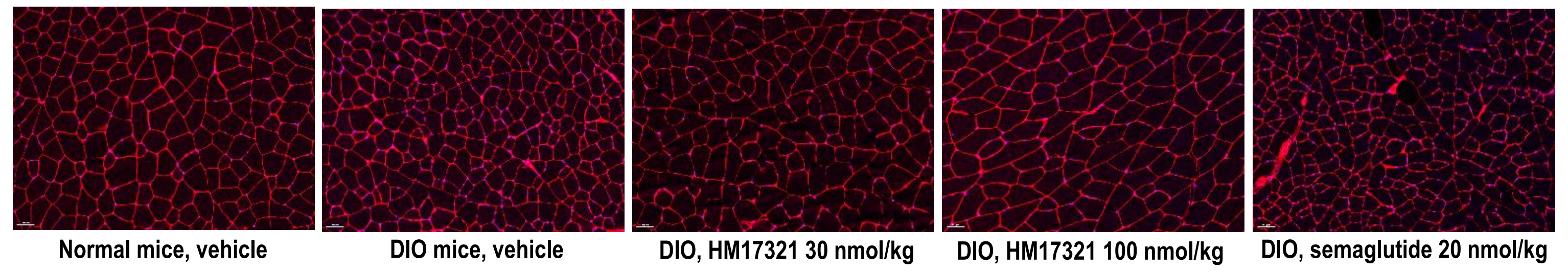
(c) TG* content in gastrocnemius (d) Quadriceps cross section area (CSA)



Note.
*IMAT: Intermuscular adipose tissue; TG: Triglyceride

*~***$p<0.001$ *vs.* DIO vehicle by One-way ANOVA test, †~††$p<0.05$~0.01 by an unpaired t-test (one-tailed)

(e) Representative images of CSA (Quadriceps)

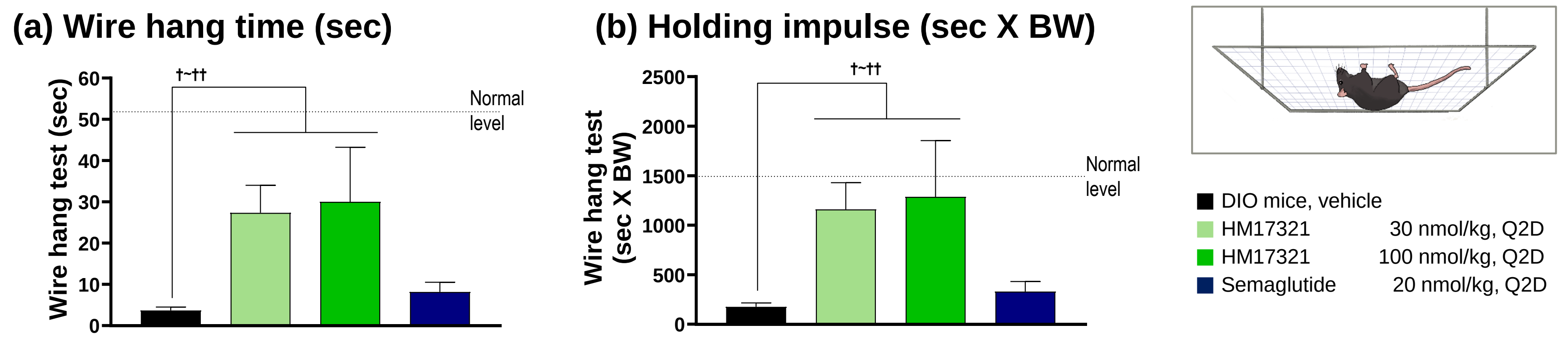


➢ In DIO mice, HM17321 dose dependently decreased BW and fat mass. Notably, unlike Sema, significant increases in lean mass and myofiber size (CSA) were observed along with muscle fat reduction. These results demonstrate HM17321 could not only reduce BW and fat mass, but also enhance muscle quantity / quality, improving WLQ.

Beneficial effect of HM17321 on muscle function

Figure 3. Effect of HM17321 on functional ability in mice

(a) Wire hang time (sec) (b) Holding impulse (sec X BW)



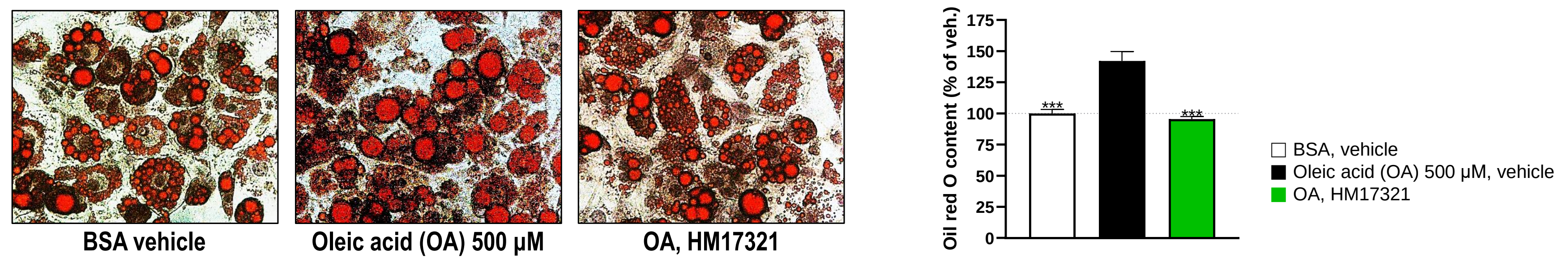
†~††$p<0.05$~0.01 by an unpaired t-test (one-tailed)

➢ In DIO mice, HM17321 effectively increased the reduced holding impulse in obese mice, indicating HM17321 contributed to improvements in muscle strength and function.

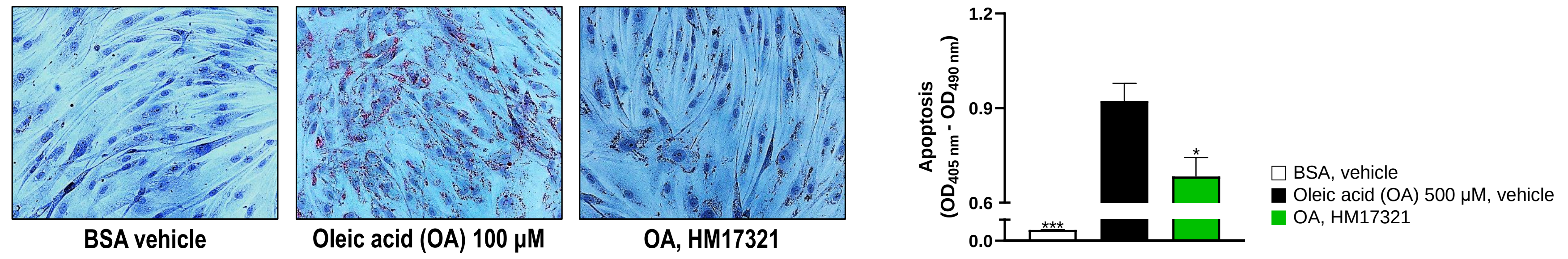
In vitro evidence for direct lipid lowering effect in adipocytes and muscle cells

Figure 4. Effect of HM17321 on adipocytes and skeletal muscle cells

(a) Representative images of Oil Red O stained 3T3-L1 (b) Quantification of Oil Red O staining



(c) Representative images of Oil Red O stained hSkMC (d) Quantitative DNA fragment analysis



*~***$p<0.05$~0.001 *vs.* OA vehicle by One-way ANOVA test

➢ In 3T3-L1 adipocytes, HM17321 prevented FFA-induced severe lipid droplet accumulation. Similarly, HM17321 reduced FFA-induced lipid accumulation and subsequent apoptosis of mature human primary muscle cell. These results propose the underlying MoAs for simultaneous fat mass reduction and muscle mass increase observed in DIO mice.

CONCLUSIONS

- **HM17321, a novel long-acting CRFR2 selective and biased UCN2 analog, is designed as a next-generation obesity treatment that selectively reduces fat mass while increasing lean mass.**
- **In DIO mice, HM17321 showed significant body fat reduction and increased muscle mass simultaneously, suggesting ideal body re-compositioning with HM17321 treatment.**
- **HM17321, but not Sema, significantly increased muscle fiber size (CSA) and holding impulse (wire hang), demonstrating that HM17321 could enhance both muscle mass and function.**
- **HM17321 directly reduced FFA-induced severe lipid accumulation in 3T3-L1 adipocytes and human skeletal muscle cells, explaining the underlying mechanisms for the favorable body re-compositioning effect of HM17321 *in vivo*.**
- **Therefore, HM17321 could be a novel and ideal therapeutic option for high quality obesity management (BW / fat mass ↓ and muscle mass ↑).**

*** Please note additional posters presenting Hanmi's incretin pipeline, a GLP-1/GIP/Glucagon triple agonist, HM15275 (Poster-335) and its COMBO w/ HM17321 (Poster-329).**